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Monitoring Indoor Exposure to Organophosphate Flame Retardants: Hand Wipes and House Dust

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Abstract

Background: Organophosphate flame retardants (PFRs) are becoming popular replacements for the phased-out polybrominated diphenyl ether (PBDE) mixtures, and are now commonly detected in indoor environments. However, little is known about human exposure to PFRs since they cannot be easily measured in blood or serum.

Objectives: To investigate relationships between the home environment and internal exposure, we assessed associations between two PFRs, tris(1,3-dichloropropyl) phosphate (TDCIPP) and triphenyl phosphate (TPHP), in paired handwipe and dust samples, and concentrations of their metabolites in urine samples (n=53). We also assessed short-term variation in urinary metabolite concentrations (n=11 participants; n=49 samples).

Methods: Adult volunteers in North Carolina, USA, completed questionnaires and provided urine, handwipe, and household dust samples. PFRs and PBDEs were measured in handwipes and dust. Bis(1,3-dichloropropyl) phosphate (BDCIPP) and diphenyl phosphate (DPHP), metabolites of TDCIPP and TPHP, were measured in urine.

Results: TDCIPP and TPHP were detected frequently in handwipes and dust (>86.8%), with geometric mean concentrations exceeding those of PBDEs. Unlike PBDEs, dust TDCIPP and TPHP levels were not associated with handwipes. However, handwipe levels were associated with urinary metabolites. Participants with the highest handwipe TPHP mass, for instance, had DPHP levels 2.42 times those of participants with the lowest levels (95% confidence interval: 1.23, 4.77). Women also had higher levels of DPHP, but not BDCIPP. BDCIPP and DPHP concentrations were moderately to strongly reliable over five consecutive days (intraclass correlation coefficient=0.81 and 0.51, respectively).

Conclusions: PFR exposures are widespread and hand-to-mouth contact or dermal absorption may be important pathways of exposure.

Introduction

Consumer products and construction materials are frequently treated with flame retardants (FRs) to reduce their flammability and meet fire safety standards. Historically, polybrominated diphenyl ethers (PBDEs) were used as the primary FRs in polyurethane foam and electronics. However, concern over the persistence, bioaccumulation, and toxicity of PBDEs led to regulatory actions and drastic reductions in their use beginning in the mid-2000s. During the same period, the use of alternative flame retardants increased, allowing manufacturers to maintain compliance with fire safety standards and regulations (Stapleton et al. 2012b; van der Veen and de Boer 2012). Organophosphate flame retardants (PFRs), such as triphenyl phosphate (TPHP) and tris(1,3-dichloropropyl) phosphate (TDCIPP), are now among the most commonly used PBDE alternatives in consumer products containing polyurethane foam (Stapleton et al. 2011; Stapleton et al. 2012b; van der Veen and de Boer 2012). In our previous work, for example, we found that TDCIPP was the most commonly detected flame retardant in polyurethane foam samples taken from both baby products (Stapleton et al. 2011) and from residential furniture purchased after 2005 (Stapleton et al. 2012b).

Like their PBDE predecessors, PFRs are added during the manufacturing process and are not chemically bound to the products in which they are used, allowing them to escape into the environment over time. TDCIPP and TPHP have been ubiquitously detected in household, office, and automobile dust samples, suggesting that the general population comes into contact with these chemicals frequently (Carignan et al. 2013; Meeker and Stapleton 2010; Stapleton et al. 2009). Our previous work examining pathways of human exposure to PBDEs indicates that exposure to contaminated dust is associated with higher body burdens, and that hand-to-mouth behaviors may be an important pathway by which PBDEs enter the body (Stapleton et al. 2012a;

Watkins et al. 2011). It remains unclear if these relationships also apply for PFRs, although correlations between the levels of TDCIPP in dust and its primary urinary metabolite (bis(1,3-dichloropropyl) phosphate (BDCIPP)) have been reported (Carignan et al. 2013; Meeker et al. 2013). Here, we examine relationships between TDCIPP and TPHP concentrations in the home environment and internal exposure using concurrent measures in handwipes and household dust, and measures of their metabolites in urine (i.e. BDCIPP and diphenyl phosphate (DPHP), respectively). Additionally, we examined associations between urinary metabolite levels and demographic (e.g. age and gender) and personal habits (e.g. hand washing behavior) to determine their potential influence on exposure. Lastly, we sought to compare levels of PFRs in house dust and handwipes to the levels of PBDEs measured in the same samples.

Methods

Study design

Healthy adult volunteers were recruited to from the general population to the National Institute of Environmental Health Sciences Clinical Research Unit (CRU) in 2012 (n=64) using study flyers and word of mouth. Eligible participants were at least 18 years of age and had never been diagnosed with a kidney problem (not including kidney stones). One group of volunteers (paired sample group; n=53) was asked to completed demographic and behavioral questionnaires and provide spot urine samples at the CRU, and collect dust samples in their homes. A second group of participants (n=11) was asked to provide daily spot urine samples at the CRU on five consecutive days. All study protocols were approved by the NIEHS Institutional Review Board and all participants gave informed consent prior to providing information or samples.

Questionnaires

Participants provided information on their personal characteristics, including age, sex, race, and height and weight, which were used to calculate body mass index (BMI). Participants also completed a questionnaire designed to obtain information about their personal habits such as the average number of hours spent active in the home and the average number of times participants washed their hands per day. Information on hand washing was collected as never, 1-2 times/day, 3-5 times/day, 6-8 times/day, and >8 times/day. For analyses we collapsed hand washing into two categories: <8 times/day (low hand washing) and ≥8 times/day (frequent hand washing), with the categorizations determined based on the distribution of responses in our study population. The frequency of hand sanitizing gel use was also obtained, and participants were classified as hand gel users, or never hand gel users. Response categories for the average time spent active in the home and the average time spent driving each day were also dichotomized for analyses (≤8 hours/day and >8 hours/day for time active in the home; ≤1 hour/day and >1 hour/day for driving time).

Dust collection

Each participant was provided with instructions and a kit for the collection of household dust. Participants were instructed to insert a nylon dust collection thimble into the hose attachment of their vacuum cleaner, similar to the method used in our previous study (Stapleton et al. 2012a). Then, they vacuumed the floor in the main living area of their home for exactly two minutes (over any type of flooring). The thimble was then removed from the vacuum, sealed in a plastic bag, and returned to the CRU. The nylon thimbles were never in contact with the plastic bag. Upon receipt in the lab, the thimbles were removed, the dust sieved to <500 microns, and then

stored in amber glass vials at room temperature until analysis (n=49; 4 participants did not provide dust samples).

Handwipe collection

Handwipes samples were collected by CRU staff (wearing gloves) using previously described protocols (Stapleton et al. 2008). Briefly, for each participant a sterile gauze wipe was soaked in 3.0 mL isopropyl alcohol and the entire surface of their hands was wiped two times from the fingers to the wrist. Wipes (n=53) were sealed in individual plastic bags and were then stored at -20 degrees Celsius until analysis. Field blanks (n=5) were also collected to examine potential background contamination in the clinic.

Urine collection

Study participants provided spot urine samples during visits to the CRU (visits conducted between 0830 and 1630 hours). Urine samples were collected in standard polypropylene specimen containers and were stored -20 degrees Celsius until analysis. All participants in the paired sample group provided urine samples (n=53) and participants providing repeated samples contributed a total of 49 samples (from 11 participants).

Dust and handwipe sample processing

Handwipe and dust samples were extracted in the laboratory and analyzed for brominated and organophosphate FRs including BDE-47, -99, -100, -153, -154, -209, TPHP, and TDCIPP. Each handwipe sample was extracted using a Soxhlet apparatus. Prior to Soxhlet extraction, each sample was spiked with four internal standards, d_{15}-TDCIPP (155 ng), d_{15}-TPHP (100 ng) a monofluorinated tetrabrominated diphenyl ether (F-BDE-69; 50 ng) and ^{13}C-BDE-209 (100 ng) (Stapleton et al. 2014). To serve as laboratory blanks, three new sterile gauze pads were taken

through the same procedure and run next to the handwipe samples. After Soxhlet extraction, each extract was concentrated using an automated nitrogen evaporation system (Turbo Vap II, Zymark Inc.) and transferred to a 4.0 mL amber vial, stored in a -20 degrees Celsius freezer. Extracts were then cleaned using Florisil solid-phase extraction (Supelclean ENVI-Florisil, 6mL, 500mg bed weight, Supelco), eluting the F1 fraction with 10mL hexane (PBDEs) and the F2 fraction with 10mL ethyl acetate (PFRs), based on the method developed by Van den Eede et al. (Van den Eede et al. 2012). Each fraction was concentrated to approximately 1mL using a nitrogen concentration system and transferred to an autosampler vial (ASV) for gas chromatography–mass spectrometry (GC/MS) analysis (Stapleton et al. 2014). Dust samples (~100 mg) were extracted with 10 mL of 50:50 dichloromethane (DCM):hexane using sonication. This process was repeated three times and the combined extract (~30 mL) was concentrated using an automated nitrogen evaporation system (Turbo Vap II, Zymark Inc.) and transferred to a 4.0 mL amber vial, stored in a -20 degrees Celsius freezer. The dust extracts were cleaned using the same method as described for the handwipe samples above. To measure recovery of the brominated internal standards, the extracts were spiked with 2, 2', 3, 4, 5, 5'-hexachloro[13C12] diphenyl ether (^{13}C-CDE 141), while d_9-TCEP was spiked into each sample to measure recovery of d_{15}-TDCIPP and d_{15}-TPHP. Recoveries of F-BDE-69, ^{13}C-BDE-209, d_{15}-TDCIPP and d_{15}-TPHP averaged 91 ±18%, 63 ±17% 75 ±11% and 75 ±7%, respectively, in all samples. Analysis of laboratory blanks (n=5) and an indoor dust Standard Reference Materials (SRM 2585, NIST, Gaithersburg, MD) were also employed for quality assurance and quality control. FR measurements in handwipes were blank subtracted using the average mass of FR measured in the field blanks. Method detection limits were calculated using three times the standard deviation of the appropriate blank (i.e. dust or handwipe). MDLs for the PFRs ranged

from 0.6 ng/g for TPP to 20.0 ng/g for TDCPP in dust laboratory blanks. In handwipes, MDLs ranged from 10 to 15 ng for the PFRs. Measured PBDE levels in SRM 2585 ranged from 78 to 130 % of certified values. Measurements of TPHP and TDCIPP in SRM 2585 were 520 ± 34, and 1820 ± 90 ng/g, respectively. These values are very similar to reports published by Van den Eede et al. (van den Eede et al. 2011), and Bergh et al. (Bergh et al. 2012).

Urine processing and analysis

Urine samples were assessed for the primary metabolites of TDCIPP and TPHP, BDCIPP and DPHP, respectively (Cooper et al. 2011). Cooper et al. (2011) provides a detailed description of the methods for extraction and measurement of BDCIPP and DPHP in urine. Briefly, BDCIPP and DPHP were measured using mixed-mode anion exchange solid phase extraction and a mass-labeled internal standard (d10-BDCIPP and d10-DPHP) with analysis by atmospheric pressure chemical ionization liquid chromatography tandem mass spectrometry (Cooper et al. 2011). We evaluated the recovery of d10-BDCIPP and d10-DPHP in all samples, and measured the amount of BDCIPP and DPHP levels in laboratory blanks (n=5) for quality assurance purposes. Average recoveries of d10-BDCIPP and d10-DPHP were 78 ± 20 and 82 ± 4%, respectively. Very small amounts of DPHP were detected in laboratory blanks, while BDCIPP was not detected. Therefore, the method detection limit (MDL) was calculated using three times the standard deviation of the blanks normalized to the urine volume extracted. To account for urine dilution, specific gravity (SG) was also measured in each urine sample prior to analysis using a digital handheld refractometer (Atago, Bellevue, WA, USA). Creatinine, an alternative means of adjusting for dilution, was not measured in samples, as it is known to vary considerable by age and gender (James et al. 1988).

Statistical analyses

We imputed concentrations below the MDL as the MDL/√2 in statistical analyses. For congeners that were detected in greater than 70% of samples, we calculated Spearman correlation coefficients to determine the associations between continuous household dust, handwipes, and urine levels (BDCIPP and DPHP only). Our preliminary investigations indicated that PFR, PBDE, and PFR metabolite concentrations were log-normally distributed and therefore, $\log_{10}$-transformed values were used in all other statistical analyses.

We used linear regression models to determine predictors of continuous levels of PFRs and PBDEs in handwipes and PFR metabolites in urine samples (continuous outcome measures were $\log_{10}$-transformed). To aid in the interpretation of results, we exponentiated beta coefficients (10^{β}), producing the multiplicative change in outcome. As predictors of congener levels in handwipes, dust concentrations were categorized into tertiles, and as predictors of urinary PFR metabolites, both dust and handwipe concentrations were categorized to minimize the effect of skewed data and outliers in regression analyses.

As a measure of temporal reliability of BDCIPP and DPHP in urine, we calculated the intraclass correlation coefficients (ICCs) and 95% confidence intervals (CI) (Hamer 1995; Shrout and Fleiss 1979). ICCs provide a measure of the reliability of repeated measures over time and are calculated by taking the ratio of the between-subject variability to the sum of the between- and within-subject variability (Rosner 2000). Additionally, to determine whether the correlations between time points deteriorated over time, we assessed Spearman correlations between each set of time points (e.g. time 1/time 2 and time 1/time 3). Statistical analyses were performed in SAS (version 9.2; SAS Institute Inc, Cary, NC), with statistical significance defined as α=0.05.

To investigate the impacts of differences in urine dilution on results we conducted analyses of urinary metabolites using raw BDCIPP and DPHP measures and also conducted analyses using specific gravity corrected concentrations (Boeniger et al. 1993). Three participants had very dilute urine (SG<1.005). Measured levels of BDCIPP and DPHP were non-detectable for these participants; however, accounting for urinary dilution resulted in large corrected value estimates. As there was substantial uncertainty around these estimate concentrations, we excluded these participants from analyses investigating the impact of SG correction. Results using each method were very similar. As such we have chosen to present uncorrected analyses including all participants.

Results

Of the 53 adults that completed demographic and behavioral questionnaires, approximately half were male (49.1%), and the majority reported white race (75.5%) and non-Hispanic ethnicity (94.3%). Participants averaged 43.6 years of age at the time of the study (range 19-67).

TDCIPP and TPHP in dust

TDCIPP and TPHP were detected in all dust samples collected in participants' homes (Table 1). Levels of TDCIPP and TPHP were highly variable in house dust, with the highest concentrations being 200- and 400-fold greater than the lowest concentrations, respectively. BDE-47, -99, -100, -153, -154, and 209 were also detected frequently in dust samples (≥87.5% detect for all congeners). With the exception of BDE-209, the geometric mean concentrations of TDCIPP and TPHP were greater than those of the individual PBDE congeners assessed; however, levels of TDCIPP and TPHP were comparable to the sum of the pentaBDE congeners which were used in similar applications to PFR flame retardants until the early 2000s (i.e. the sum of

BDE-47, -99, -100 and -153; GM pentaBDE=1117.8 ng/g; Stapleton et al. 2009). TDCIPP concentrations in dust were significantly correlated with PBDE congeners in dust (r_s: 0.50 to 0.57; Table 2). Levels of TPHP and BDE-47, -100, and -209 in dust were also correlated (r_s: 0.37, 0.33, and 0.29, respectively), although the magnitudes of correlations were lower than for TDCIPP.

TDCIPP and TPHP in handwipes

TDCIPP and TPHP were also detected frequently in handwipe samples (90.6% and 86.8%, respectively; Table 1). Geometric mean concentration of TDCIPP and TPHP on participants' hands exceeded the levels of individual PBDE congeners, which were also detected in nearly all handwipe samples. TDCIPP and TPHP were moderately correlated with each other in handwipes (r_s: 0.42, p=0.002; Table 2). The levels of TDCIPP on participants' hands were correlated with the levels of BDE-47, -99, -100, -153, and -154 on handwipes. Although the levels of PBDEs in house dust and handwipes were moderately correlated (r_s: 0.33 to 0.49), TDCIPP and TPHP levels were not correlated between the two matrices (Table 2). We used linear regression models with categorized dust concentrations to further explore relationship between FRs in handwipes and dust. As in the correlation analyses, we did not observe evidence of associations between the levels of TDCIPP or TPHP on participants' hands and the levels in household dust (Table 3). Increasing levels of PBDEs in house dust, however, were strongly associated with their levels on handwipes. For example, those with the highest dust levels (3rd tertile) of BDE-100 in their homes averaged 3.44 times (95% confidence interval (CI): 1.25, 9.44) the levels of BDE-100 in handwipe samples compared to those with the lowest dust levels (Table 3).

We also investigated associations between demographic and behavioral information and the levels of flame retardants in handwipes using linear regression models. Associations were

generally imprecisely estimated and did not follow a consistent pattern across FRs (Supplemental Material, Table 1). For example, our results suggested inverse associations between hand washing frequency (<8 times/day vs. ≥8 times/day) and TDCIPP, BDE-47, -99, -100, -153 and -154 handwipe levels, while frequent hand washing tended to be related to higher TPHP and -209 levels on participants' hands.

DPHP and BDCIPP in urine

DPHP and BDCIPP were detected frequently (90.6% and 83.0% detect) in urine samples from participants with paired house dust and handwipe samples, with a geometric means of 1.02 ng/mL and 0.37 ng/mL, respectively (n=53 samples). Concentrations ranged from non-detectable to 9.09 ng/mL for DPHP, and non-detectable to 4.46 ng/mL for BDCIPP. The levels of TDCIPP and TPHP in dust were not correlated with the measures of their metabolites in urine (Table 4). Spearman correlation coefficients suggested an association between the levels of TPHP in handwipes and the levels of DPHP in urine (r_s: 0.37, p=0.006; Table 4) and the levels of TDCIPP in dust and BDCIPP in urine (r_s: 0.27, p=0.06; Table 4). We conducted regression analyses using categorical versions of handwipe and house dust variables as predictors of urinary BDCIPP and DPHP to further explore these relationships. Although levels of BDCIPP and DPHP were on average higher for participants living in homes with the highest levels of TDCIPP and TPHP in dust (3rd tertile), effect estimates were imprecisely estimated and did not follow a consistent pattern across the exposure gradient (comparing the 3rd tertile to the 1st 10^{β} =1.27; 95% CI: 0.53, 3.04 and 10^{β}=1.23; 95% CI: 0.57, 2.67; Table 5). Conversely, results suggest that categorical handwipe levels of TDCIPP and TPHP may be associated with levels of BDCIPP and DPHP in participants' urine (Table 5). Participants with the highest levels of TDCIPP on their hand, for

instance, had urinary BDCIPP levels 1.99 times those of participants with the lowest levels of TDCIPP on their hands (95% CI: 0.89, 4.47).

Several demographic and behavioral factors were also associated with the levels of PFR metabolites in urine samples. Women had significantly higher levels of DPHP in urine samples than men (10^{β}=1.84; 95% CI: 1.05, 3.21; Table 5) and levels of both BDCIPP and DPHP decreased with age (10^{β}=0.97; 95% CI: 0.94, 0.99 and 10^{β}=0.98; 95% CI: 0.95, 1.00, respectively). Participants proving samples at the CRU in the afternoon tended to have higher levels of BDCIPP and DPHP in their urine (10^{β}=2.15; 95% CI: 1.09, 4.27 and 10^{β}=1.45; 95% CI: 0.78, 2.68, respectively). Although not statically significant, results were suggestive of an inverse association between average hand washing frequency (<8 times/day vs. ≥8 times/day) and the levels of BDCIPP and DPHP in urine (10^{β}=0.57; 95% CI: 0.28, 1.14 and 10^{β}=0.90; 95% CI: 0.48, 1.68, respectively)

Temporal variation in urinary BDCIPP and DPHP

For participants with repeated urine samples, the rank order of BDCIPP and DPHP urine concentrations was similar over time (Supplemental Material, Figure 1). We examined the correlations between urine measures at each time point individually using Spearman correlations and found no evidence of reduced correlations over time (e.g. the correlation between each time point was similar; data not shown). Examining temporal variability in BDCIPP levels using ICCs, we observed strong consistency over the course of five consecutive days (ICC=0.81; 95% CI: 0.75, 0.86) (Rosner 2000). DPHP levels in urine were also moderately to strongly consistent over the course of five days (ICC=0.51; 95% CI: 0.42, 0.63).

Discussion

Cumulatively, our results suggest that exposures to PFRs are common, and variable in the general adult population. We found detectable levels of TDCIPP and TPHP in nearly all house dust and handwipe samples. TDCIPP and TPHP were generally detected at levels well above those of BDE-47, -99, -100, -153 and -154. Levels of PFRs and PBDEs in dust were similar to those reported from recent studies in California and North Carolina (Dodson et al. 2012; Meeker et al. 2013; Stapleton et al. 2014). As products containing PBDEs are replaced with newer products containing alternative flame retardants, their levels may decreases. However, the levels of alternative flame retardants, such as TDCIPP and TPHP may increase over time. Dodson et al. (2012), for example, reported declining levels of PBDEs in indoor dust collected in California homes (between 2006 and 2011), and increasing levels of alternative flame retardants, including TDCIPP, reflective of changes in FR applications in residential furniture (Stapleton et al. 2012b). Additional research is needed to determine whether the levels of TDCIPP and TPHP that we observed in the indoor environment and on participants' hands impact human health.

The primary metabolites of TDCIPP and TPHP (i.e. BDCIPP and DPHP), were also detected in the vast majority of urine samples provided by study participants. Urinary DPHP and BDCIPP were approximately 3-fold higher in our current work that those reported previously in adult men (Meeker et al. 2013), similar to levels reported in office workers (Carignan et al. 2013) from the Boston, Massachusetts area, and lower than in the levels we observed in a previous investigation of pregnant central North Carolina women (Hoffman et al. 2014). Near ubiquitous detection of PFR metabolites is of particular concern as the health impacts of PFR exposures remain largely unexplored in humans, but *in vitro* and animal data suggest that they may be endocrine-

disrupting and carcinogenic (Babich 2006; Belcher et al. 2014, Farhat et al. 2013; Gold et al. 1978; Kojima et al. 2013; Liu et al. 2012 and 2013; Wang et al. 2013).

Collecting paired house dust, handwipe, and urine samples from study participants allowed us to examine associations between sample types and to explore potential pathways of exposure. We did not observe associations between measures of TDCIPP or TPHP in house dust and the levels on participants' hands. There are several possible reasons for the lack of association. For example, handwipe samples were collected at the CRU while dust samples were collected in participants' homes. It is possible that the levels of TDCIPP and TPHP on participants' hands at the CRU were more reflective of recent TDCIPP and TPHP exposure, including exposure in other microenvironments that they may have recently visited (e.g. automobiles, the work place, or the CRU). However, PBDEs in house dust were correlated with the levels on participants' hands, which suggest that contact with PBDE contaminated dust in the home environment was contributing to the levels of FRs on handwipes, despite the measurements being take at different times and locations (i.e. the home and the CRU). Differences in the physicochemical properties between PFRs and PBDEs may also explain these differences. TDCIPP, for example, is a smaller compound and has a higher vapor pressure than the PBDEs. Recent research from Weschler and Nazaroff (2012) speculated that semivolatile organic compounds (SVOCs) in indoor air may sorb to skin, suggesting that the weaker association for the PFRs between handwipes and dust may reflect a larger contribution of PFRs on handwipes from the indoor air than from house dust (Weschler and Nazaroff 2012). Similarly, Cao et al. (2014) recently demonstrated seasonal variation in the levels of PFRs in dust, but little variation in the levels of PBDEs (Cao et al. 2014).

Although dust samples were not associated with metabolites, higher levels of TDCIPP and TPHP on handwipes were significantly associated with the levels of their metabolites in urine samples. Handwipes may provide a more integrated picture of internal exposure, including information from multiple microenvironments, and may provide more biologically relevant measures of exposure than the levels of dust in a single room in the home. Although our work is the first to investigate relationships between dust and handwipe PFRs with urinary metabolites, similar associations have been reported for PBDEs, with handwipe levels being more strongly related to internal exposure than dust measures in a single microenvironment (e.g. homes or offices) (Stapleton et al. 2008; Stapleton et al. 2012a; Watkins et al. 2011). Additionally, the strong relationship between the levels of TDCIPP and TPHP on handwipes, and the levels of their metabolites in urine, suggests that hand-to-mouth contact or dermal absorption may be important pathways of exposure.

It is also interesting to note that DPHP concentrations in urine samples from women were almost twice those of men, which may suggest differences in exposure patterns by sex. For example, similar patterns have been observed for some phthalate metabolites (e.g. monobenzyl phthalate and monoethyl phthalate), a finding which has been attributed to difference in the use of personal care products between males and females (Silva et al. 2004). Alternatively, differences in the metabolism of TPHP between men and women may be driving the differences in the levels of DPHP in urine. Although TPHP is reportedly used in nail polish, we are not aware of other common personal care products in which it is used (Hagopian et al. unpublished manuscript). Similarly, we observed higher levels of BDCIPP and DPHP for participants that provided urine samples in the afternoon, suggesting differences in exposure patters throughout the day.

In vivo and *in vitro* studies suggest that TDCIPP and TPHP are rapidly metabolized (to BDCIPP and DPHP, respectively) and eliminated from the body (Cooper et al. 2011; Lynn et al. 1981; Nomeir et al. 1981). We observed moderate to strong reliability in the levels of BDCIPP and DPHP in urine samples collected on five consecutive days. The observed ICCs (BDCIPP=0.81 and DPHP=0.51) were much greater than those typically reported for rapidly metabolized compounds with primarily dietary sources (e.g. organophosphate pesticides; (Bradman et al. 2013)). Previous studies assessing the reliability of repeated measures to BDCIPP and DPHP in pregnant women and in adult men, have also reported moderate to strong reliability (3 measurements throughout pregnancy, DPHP ICC=0.5 and BDCIPP ICC=0.6 (Hoffman et al. 2014), and 9 samples over 3 months DPHP ICC =0.7 and BDCIPP ICC=0.5 (Meeker et al. 2013)). These findings suggest that TDCIPP and TPHP may come from more continuous sources of exposure, such as contact with products containing PFRs or contact with contaminated dust. Nonetheless, variation in daily behavior (e.g. working in an office environment or spending more time at home), may impact levels of exposure to PFRs.

Our study has several limitations that should be considered in the interpretation of results. Paired dust, handwipe, and urine samples were each collected only once. Multiple samples taken over time and in different micro environments (e.g. workplaces and cars) may provide additional insights as to important routes of exposure to PFRs. Similarly, we did not measure the concentrations of TDCIPP or TPHP in indoor air. Both TDCIPP and TPHP have been detected in household air samples previously (Staaf and Ostman 2005), and data suggest that inhalation exposure may be an important pathway to consider in future assessments (Stapleton et al. 2009). In addition, although detailed instructions were provided, household dust samples were collected by participants; variability in the areas sample and the types of vacuums used may have

introduced measurement error into our analyses. We expect any measurement error introduced by differences in dust collection between participants was not related to the levels of TDCIPP or TPHP in house dust and therefore, may have biased our result toward the null. Additionally, our small sample size limited the number of predictive variables that we could include in multivariate regression analyses at the same time and may have limited our power to detect meaningful associations. Finally, although participants were recruited from the general North Carolina population, the cohort was comprised of a relatively homogeneous group; participants were primarily white and there was little variability in behavioral characteristics. Although this may limit our ability to generalize results to the broader US population, it does not impact the internal validity of our results.

Conclusions

Cumulatively, our results indicate that PFR exposures are widespread in the general adult population. Hand-to-mouth contact or dermal absorption may be important pathways of exposure as the levels of TDCIPP and TPHP on handwipes are associated with the levels of their metabolites in urine. Our results suggest that handwipe measures of TDCIPP and TPHP may provide a means of characterizing exposure to PFRs in future epidemiologic studies. Such studies are needed to determine whether the levels of TDCIPP and TPHP that we observed in the indoor environment impact human health, particularly as animal studies suggest that PFRs may adversely impact health.

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Table 1. Geometric mean and range of flame retardants in household dust (n=49) and handwipes (n=53) collected for North Carolina adults.

Congener	Dust (ng/g)			Handwipes (ng)		
	% Detect	GM[a]	Range	% Detect	GM[a]	Range
TDCIPP	100.0	1390	197-39,530	90.6	84.1	ND[b]-537
TPHP	100.0	1020	99.5-40,350	86.8	62.1	ND[b]-1,230
BDE47	100.0	374	28.4-21,800	100.0	18.4	2.5-454
BDE99	100.0	510	29.8-17,280	100.0	26.0	4.4-707
BDE100	100.0	128	19.3-4,702	81.1	2.8	ND[b]-128
BDE153	91.7	52.2	ND[b]-2,609	90.6	1.3	ND[b]-67.9
BDE154	87.5	45.5	ND[b]-1,969	86.8	1.0	ND[b]-59.8
BDE209	100.0	1280	103-44,900	96.2	19.5	ND[b]-804

[a]GM: geometric mean. [b]ND: non-detect

Table 2. Correlation matrix for flame retardants levels measured in paired handwipes and household dust. Correlation analyses were conducted on dust and handwipe data in which detection frequency was >70%. Shaded correlations indicate relationships between the same congener measured in dust and handwipes.

		Dust								**Handwipes**							
		TDCIPP	TPHP	BDE47	BDE99	BDE100	BDE153	BDE154	BDE209	TDCIPP	TPHP	BDE47	BDE99	BDE100	BDE153	BDE154	BDE209
Dust	TDCIPP	1.00															
	TPHP	0.17	1.00														
	BDE47	0.50†	0.37#	1.00													
	BDE99	0.54†	0.22	0.90†	1.00												
	BDE100	0.55†	0.33*	0.96†	0.94†	1.00											
	BDE153	0.57†	0.23	0.88†	0.90†	0.95†	1.00										
	BDE154	0.56†	0.26	0.92†	0.93†	0.98†	0.97†	1.00									
	BDE209	0.54†	0.29*	0.34*	0.31*	0.41#	0.42#	0.44†	1.00								
Handwipes	TDCIPP	0.10	-0.05	-0.07	-0.13	-0.09	-0.06	-0.06	-0.03	1.00							
	TPHP	-0.09	0.18	-0.12	-0.15	-0.14	-0.21	-0.17	-0.10	0.42#	1.00						
	BDE47	0.17	0.15	0.38#	0.34*	0.38#	0.38#	0.37#	0.11	0.39#	0.32*	1.00					
	BDE99	0.27	0.23	0.47†	0.43*	0.46†	0.49†	0.46†	0.20	0.32*	0.21	0.88†	1.00				
	BDE100	0.11	0.12	0.40*	0.34*	0.41#	0.43#	0.41#	0.13	0.33*	0.22	0.89†	0.85†	1.00			
	BDE153	0.27	0.10	0.40#	0.38#	0.42#	0.49†	0.46†	0.20	0.40#	0.20	0.84†	0.88†	0.88†	1.00		
	BDE154	0.22	0.17	0.38#	0.36*	0.39#	0.43#	0.41#	0.22	0.35*	0.06	0.83†	0.85†	0.87†	0.86†	1.00	
	BDE209	-0.03	0.12	0.17	0.24	0.18	0.16	0.20	0.33*	0.16	0.06	0.20	0.20	0.14	0.20	0.13	1.00

*<0.05
#<0.01
†<0.001

Table 3. Regression analyses for dust congener levels as predictors of handwipe FR levels. Analyses were conducted on dust and handwipe data in which detection frequency was >70%.

Flame Retardant	Low	Mid		High	
		Coefficient[a] (95% CI)	P-value	Coefficient[a] (95% CI)	P-value
TDCIPP	Reference	0.90 (0.45, 1.84)	0.78	1.18 (0.59, 2.39)	0.63
TPHP	Reference	1.20 (0.51, 2.82)	0.66	1.08 (0.46, 2.54)	0.85
BDE47	Reference	1.36 (0.55, 3.37)	0.50	2.62 (1.05, 6.49)	0.04
BDE99	Reference	1.29 (0.56, 2.97)	0.55	2.45 (1.06, 5.66)	0.04
BDE100	Reference	1.61 (0.59, 4.43)	0.35	3.44 (1.25, 9.44)	0.02
BDE153	Reference	2.94 (0.99, 8.75)	0.05	5.13 (1.73, 15.22)	0.004
BDE154	Reference	2.16 (0.73, 6.41)	0.15	3.49 (1.18, 10.35)	0.03
BDE209	Reference	2.34 (0.90, 6.06)	0.08	2.32 (0.89, 6.01)	0.08

[a]Exponentiated beta-coefficients represent the multiplicative change in urine concentrations relative to the reference group.

Table 4. Correlation matrix for flame retardants levels measured in paired handwipes (n=53), dust (n=49), and urine samples (n=53).

		Dust		Handwipes	
		TDCIPP	**TPHP**	**TDCIPP**	**TPHP**
Urine	**BDCIPP**	0.10	0.04	0.27	0.13
	DPHP	-0.17	0.15	0.17	0.37[#]

[#]<0.01

Table 5. Regression analyses for predictors of urinary BDCIPP and DPHP.

Predictor	BDCIPP		DPHP	
	Coefficient[a] (95% CI)	**P-value**	**Coefficient[a] (95% CI)**	**P-value**
Sex				
Male	Reference	--	Reference	--
Female	1.00 (0.51, 1.95)	0.99	1.84 (1.05, 3.21)	0.03
Age (years)	0.97 (0.94, 0.99)	0.008	0.98 (0.95, 1.00)	0.03
Visit time				
Morning	Reference	--	Reference	--
Afternoon	2.15 (1.09, 4.27)	0.03	1.45 (0.78, 2.68)	0.23
Average times hands washed				
<8 times/day	Reference	--	Reference	--
≥8 times/day	0.57 (0.28, 1.14)	0.11	0.90 (0.48, 1.68)	0.74
Handgel use				
No	Reference	--	Reference	--
Yes	0.95 (0.46, 1.94)	0.89	0.74 (0.40, 1.38)	0.34
Average time active in the home				
≤8 hours/day	Reference	--	Reference	--
>8 hours/day	1.46 (0.68, 3.15)	0.16	1.23 (0.63, 2.42)	0.54
Average time driving in car				
≤1 hour/day	Reference	--	Reference	--
>1 hour/day	0.63 (0.32, 1.21)	0.16	0.81 (0.46, 1.46)	0.48
Dust TDCIPP or TPHP levels				
Low	Reference	--	Reference	--
Mid	0.91 (0.38, 2.17)	0.67	0.86 (0.40, 1.87)	0.70
High	1.27 (0.53, 3.04)	0.72	1.23 (0.57, 2.67)	0.59
Handwipe TDCIPP or TPHP congener levels				
Low	Reference	--	Reference	--
Mid	1.51 (0.67, 3.39)	0.31	1.30 (0.66, 2.57)	0.44
High	1.99 (0.89, 4.47)	0.09	2.42 (1.23, 4.77)	0.01

[a]Exponentiated beta-coefficients represent the multiplicative change in urine concentrations relative to the reference group for categorical variables, or the per unit change for continuous variables (age).